12.2 LAKES

12.2.1. Bacteria

Information concerning bacteria can be found in Streams, Section 12.1.1.

12.2.2 Phytoplankton

Phytoplankton are free-living unicellular algae existing as single cells, colonies, chains, or filaments that generally are passively transported by currents and turbulent mixing. Phytoplankton are important components of standing bodies of water and large rivers.

Physiological processes of planktonic algae can indicate the productivity and quality of natural waters. Their assimilation of carbon dioxide and production of organic matter provide a primary food source for other trophic levels. Phytoplankton can also have a dramatic affect on the concentrations of carbon dioxide, oxygen, nutrients, silica and trace elements.

Phytoplankton blooms can severely effect water quality through the production of toxins that lead to fish kills, threatens human health, or through the depletion of oxygen caused by the decomposition of organic matter.

Integrated studies of aquatic ecosystems need to include the measurement of phytoplankton composition and biomass. Chlorophyll *a*, adenosine triphosphate (ATP), and particulate organic carbon or nitrogen can be used as indices of biomass while particle counters can provide information about size distribution. However, these methods have interferences from nonphytoplankton particulate matter such as detritus and sediment. The only method of determining species composition and abundance is by microscopic enumeration and identification. Knowledge of species composition can indicate the causes of seasonal changes in biomass, be useful as tracers for different water masses, and suggest stresses imposed by pollutants.

12.2.2.1 Collection

An optimum method for collecting phytoplankton samples is unavailable because phytoplankton types and abundance differ spatially and temporarily. Therefore, choosing a sampling strategy and method that are most consistent with the goals of a given water is necessary for a quality study. For example, frequent collection of a depth-integrated sample at one representative site may be appropriate for a monitoring study, whereas, a detailed grid may be more appropriate for assessing the effect of a pollutant from a point source.

A phytoplankton sample consists of a volume of water (usually 100-1000ml) collected in a polyethylene bottle. To insure maximum correlation of results, the sample site and collection method need to correspond as closely as possible to those selected for chemical sampling. If a living sample is to be examined, it can be maintained for 24 hours if kept at 3°C in the dark. Extended storage requires preservation with 1ml of Lugol's solution (a mixture of potassium iodine, iodine, and acetic acid).

Samples of phytoplankton are collected using a water-sampling bottle (Kemmerer or Van Dorn) and a depth-integrated sampler (DH-48), net, or pump. An advantage of using a water-sampling bottle is the ability to collect quantitative samples including ultraplankton from a known volume obtained from a precise depth.

Depth-integrated samplers are quantitative samplers used to collect a representative of a water column cross-section. An advantage of the depth-integrated sampler is similar to the water-sampling bottle; the difference is it is the only means of collecting a truly representative sample within a water column or river cross-section. The disadvantage to the water sampling-bottle and depth-integrated sampler is that they do not work well in fast moving water. However, fast-moving water does not typically support true phytoplankton communities.

Plankton nets have been widely used as sampling devices in phytoplankton investigations because they enable filtration of large water volumes. However, nets selectively retain only the largest phytoplankton cells. Therefore, nets are most appropriate for qualitative study of large planktonic algae. Nets vary in size, shape, and mesh size.

Pumps can also be used to collect phytoplankton. The advantage of pumps is that they can collect quantitative samples including ultraplankton rapidly at known depths or via depth integration in shallow or deep waters. However,

pumps are usually bulky, expensive, require a power source, may break algal chains and colonies, and induce physiologically stresses.

12.2.2.2 Sample Analysis

Analysis of phytoplankton may include the counting-cell method where aliquots from phytoplankton samples are placed in stage-counting cells and examined under a microscope to determine concentration and possible identification. The inverted-microscope method enables the observation of phytoplankton in an aliquot of water at high-power magnification. The phytoplankton have been concentrated by settling thereby reducing the possibility of crushing or disruption. Phytoplankton should be both counted and identified. One of the most useful methods to determine the taxonomy of the phytoplankton is through the preparation of a permanent slide mount and examination under a high powered microscope.

A. REFERENCES

Methods for Collection and Analysis of Aquatic Biological and Microbiological Samples, Techniques of Water-Resources Investigations of the United States Geological Survey, Book 5 Chapter A4 (1989), at 99-113.

Standard Methods For Examining Water and Wastewater, 18th Edition (1992), at 10-1 to 10-26.

12.2.3 Trophic Status

Lake productivity or trophic status is a continuum varying from very low productivity in oligotrophic lakes (i.e., mountain lakes) to high productivity in eutrophic lakes (i.e., lowland lakes). The condition between oligotrophic and eutrophic is called mesotrophic. The boundaries around the mesotrophic status are not distinct. A method for identifying lake tropic status (TSI) has been described by Carlson (1977) using total phosphorus (TP), Secchi disk depth (SD), and Chlorophyll *a*. The approximate TSI value between oligotrophic and mesotrophic is 35, and between mesotrophic and eutrophic is 50.

Calculations to determine Carlson's Trophic State Indices:

Total Phosphorus (TP)

$$TSI = 10(6 - Ln[48/TP]/ Ln[2])$$

Secchi Disk (SD)

$$TSI = 10(6 - Ln[SD]/ Ln[2])$$

Chlorophyll *a* (Chla)

$$TSI = 10(6- 2.04 - 0.68 Ln[Chla]/ Ln[2])$$

12.2.3.1 Secchi Depth

One of the major diagnostic tools in the analysis of eutrophication is the measurement of water transparency. Algal blooms decrease light penetration by light absorption, and scattering water transparency and light penetration are proportional to the density of the algal bloom.

A simple method of estimating light penetration in the vertical direction is with a Secchi disk, where the disappearance depth is defined as the Secchi depth. The Secchi disk is submerged into the water from the shady side of an anchored boat or from the end of a pier. The Secchi disk is lowered to a point where it is no longer visible and then raised to a level where it again becomes visible. The Secchi Depth at this point is measured (meters) and recorded.

12.2.3.2 Chloriphyll *a*

Field Collections

A. Samples are collected with a 1 liter plastic bottle 0.3m (1ft) beneath the water surface.

B. Chlorophyll *a* samples are filtered through a glass fiber filter with a hand-operated vacuum pump. Enough water is filtered until the filter is green in color. At least 1 liter of water should be filtered for eutrophic lakes and up to 5 liters of water should be filtered for oligotrophic lakes. The volume of water filtered is recorded for later use in calculating Chlorophyll concentrations. Equipment to be used for filtration includes the following (Fisher Scientific catalogue numbers are given):

1. Whatman glass fiber filter GF/C 47mm Cat. No. 09-874-34

2. Nalgene hand-operated vacuum pump Cat. No. 01-070

3. Nalgene reusable filter holders Cat. No. 09-740-23E

C. The filter is then removed from the filter holder with a forceps, placed in a petri dish, taped shut, wrapped in aluminum foil and stored on ice. Samples should be shipped to the laboratory within 48 hours of collection and frozen until analysis. The analysis should be performed no later than 3 weeks after collection.

Laboratory Analysis

A. The laboratory shall analyze the Chlorophyll *a* following the "Standard Methods for Examination of Water and Waste Water", 18th Edition, Method 10200 H. Chlorophyll. This method extracts the chlorophyll from the filter using acetone and a tissue grinder. The absorbance of the extract is determined with a spectrophotometer (Method 2.c, Spectrophotometric Determination of Chlorophyll ; Determination of Chlorophyll *a, b*, and *c* (trichromatic method)).

B. Following the equations in the cited reference, the Chlorophyll *a* concentration is calculated by using the concentration of pigment in the extract and the recorded volume of water filtered in the field.

Tropic Status of Montana Lakes

A.	Oligotrophic	0 - 2ppb Chlorophyll *a*
B.	Mesotrophic	2 - 9ppb Chlorophyll *a*
C.	Eutrophic	9 - 30ppb Chlorophyll *a*
D.	Hypereutrophic	> 30ppb Chlorophyll *a*

12.2.4 Periphyton

Periphyton are algae that live attached to or in close proximity of the lake bottom in shallow, well-lighted areas that are suitable for plant growth. Other plants may also occupy the littoral areas of lakes, notably "higher plants" and mosses (macrophyton). These following procedures address the wadeable, nearshore portion of lakes; no special equipment (i.e., SCUBA) is presumed. Refer to Section 12.1.2 for more background information about periphyton.

A. INDEX PERIOD

Anytime during the ice-free season is suitable for sampling periphyton from lakes. Assessments should be delayed for at least two weeks following the melting of ice in the spring to allow for recolonization by algae and the succession to a mature periphyton community.

B. SITE SELECTION

Selection of study sites depends largely on the objectives of the assessment. Public access shall often dictate the choice of sampling sites on Montana lakes and restrict the length of shoreline that is available for sampling. If access is unrestricted, choose least-impaired sites that have typical shoreline vegetation to represent the reference or control condition. Avoid the windward side of lakes where wave action tends to scour shoreline vegetation.

12.2.4.1 Field Observations

The general composition, amount, color, and condition of aquatic plants in the littoral zone may be assessed from shore using the Aquatic Plant Field Sheet (Section 21.1.1.8). This information shall help to describe the health and productivity of the aquatic ecosystem, define nuisance aquatic plant problems, identify potential sources and causes of pollution, and document changes in the plant community over time.

Completing the Aquatic Plant Field Sheet is equivalent to a RBP Level I assessment for aquatic plants. The Aquatic Plant Field Sheet should be filled-out before completing the more detailed assessments of periphyton standing crop, composition, and community structure.

12.2.4.2. Standing Crop

The standing crop of periphyton in lakes is controlled by a variety of factors. Heavy growths of algae in lakes generally indicate nutrient enrichment and inferior water quality. Refer to Section 12.1.2.2 for sampling and analytical procedures. Criteria have not been developed for the standing crop of lakeshore periphyton.

12.2.4.3 Composition and Structure

The procedures for sampling and analyzing stream periphyton (Section 12.1.2.3) may also be used for evaluating the composition and structure of lake periphyton. In lakes, however, the submerged stems and leaves of higher plants or macrophytes shall generally be more important as algal substrates than they are in streams. The DEQ is developing biocriteria and bioassessment protocols for the composition and structure of lake periphyton.

12.2.5 Macroinvertebrates

Benthic macroinvertebrates are animals inhabiting the substratum of lakes, streams, and wetlands. Macroinvertebrates are considered, by definition, to be visible to the unaided eye and are retained on a U.S. Standard No. 30 sieve (0.595-mm openings).

12.2.5.1 Sampling Methods (Ekman Grab, Petite Ponar Grab, And D-Net)

Dredge (Grab)

A tall-model (6"x6"x9") Ekman-type grab is preferred for lake benthos sampling rather than the standard (6"x6"x6") Ekman grab. The standard Ekman grab is a poor choice for sampling macroinvertebrates in the soft sediments of deeper lakes because the box often sinks below the sediment surface and organisms in the surficial sediment are lost. Even with a tall Ekman, care must be taken that the grab does not sink too deeply and overfill. There should be water in the top of the box upon retrieval; if there is only sediment, some material was probably lost out of the top due to over-penetration. If this is a problem, horizontal struts can be added to the outside of the grab to slow the decent of the sampler into the sediment. Pieces of wood (2"x2"x18") may be attached across the outside of the box at about half-height for this purpose. The Ekman grab is also a poor choice for sampling when rocky or sandy bottoms, or moderate macrophyte growth are present because small pebbles or grit or macrophyte stems prevent proper jaw closure.

Some Ekman grabs have been fitted with a screen across the top to prevent overfilling. This works, but care must be taken that the grab is eased gently into the soft sediments of deep lakes. Since water flow through the box is impended by the screen, a pressure wave can blow away surficial sediment and animals if such a grab is dropped too rapidly.

An alternative to the Ekman grab is the petite Ponar grab. Standard-model Ponar grabs are too heavy for use without a winch, but the petite model is very easily cast and retrieved by hand from a small boat. DEQ has found this to be the most convenient grab for survey work, because it is easy to operate and closes automatically, needing no messenger. Its disadvantages are : 1) it has the potential for sediment blow-away if dropped too quickly into a soft bottom, and 2) it collects a smaller volume of sediment, thus fewer animals, than a tall Ekman. Therefore, the petite Ponar grab is most suitable for studies that do not require absolute quantitative data as they are likely to have greater consistency when collected by different personnel.

D-Net (Sweep Net)

For D-net sampling in the littoral zone, refer to Wetlands, Sweep Net (Section 12.3.4.1).

12.2.5.2 Sample Processing and Analysis

Traditionally grab samples are washed in a tray or bucket with a mesh bottom. However, this method is slow and removal of the washed sample is inconvenient. An easier method of washing the contents of the grab is through a conical plankton net of 0.5mm mesh (500 microns).

It is very important that the mesh size be consistent across all lakes in the study. This is a fairly standard mesh size that shall retain most of the animals of interest, but shall permit reduction of samples to a reasonable volume. A conical washing net provides a large surface area of mesh, and concentrating the sample in the end of the net is easy. A suitable net can be purchased from Wildco as a made-to-order net #30-D60. The 4.7cm (12in) diameter top opening of this net must be lashed to a hoop, such as Wildco #7-D30 ring and bridle assembly, or any rigid 4.7cm (12in) diameter ring. The cuff at the bottom of the net can be tied off with a shoestring, which is then released to permit removal of the washed and concentrated sample.

The content of the grab is emptied into a plastic dish pan using a wash bottle with a wide-bore opening for rinsing the grab and wash net. Washed samples are collected in 1 liter wide-mouth plastic jars with tight-fitting lids. The preferred method of preserving a macroinvertebrate (especially benthos) sample from a taxonomic identification perspective is using 10% formalin buffered with 1 teaspoon magnesium carbonate per 1 gallon concentrated formalin. However, because formalin is a known carcinogen, the DEQ recommends using 95% ethanol with a final dilution of no less than 70% when considering displacement by the sample biomass.

In addition to an external label, each sample should contain an internal label on heavy paper with the following information : lake, date, sampling device used, sampling depth, replicate number, and the collectors initials. A depth-finder and Global Positioning System are useful for locating the collection site. A hydrolab or similar device can be used to collect supporting environmental data.

List of Equipment and Supplies for Sample Collection:

a) Hydrographic map of the lake

b) Petite Ponar or other grab sampler or D-Net

c) Plastic tub

d) Wash net (0.5mm mesh)

e) Wash bottle

f) 1 liter plastic container with lids

g) Paper labels and labeling tape

h) Pencils and alcohol proof markers

i) Preservative (ethanol or formalin)

j) Power ice auger (winter sampling)

k) Ice skimmer (winter sampling)

m) Depth finder

n) Boat

o) Global Positioning System

p) Hydrolab

12.2.5.3 Criteria and Assessment Protocols

The composition and density (number of individuals per unit area) of macroinvertebrates in streams, lakes, and wetlands are reasonably stable year to year in unperturbed environments. However, seasonal fluctuations associated with life-cycle dynamics of individual species may result in extreme variation at specific sites within any calendar year.

Most aquatic habitats with high quality waters and substrate conditions generally support diverse macroinvertebrate communities in which there is a balanced distribution of species among the total number of individuals present. Such communities respond to changing habitat quality by adjustment in community structure. However, many habitats are dominated by a few species. Small changes in their relative numbers may not indicate changes in water quality.

Macroinvertebrate community responses to environmental perturbations are useful in assessing the impact of point and nonpoint source pollution. Situations that may cause macroinvertebrate community structure to change include organic loading, substrate alteration, and toxic chemical pollution.

Assessing the impact of a pollutant source generally involves comparison of macroinvertebrate communities at sites influenced by pollution with those collected from adjacent unaffected sites that have similar physical habitats. The procedure includes sampling and analyzing both communities and comparing them to determine if the pollution-affected site differs from the unaffected site. For community structure, the basic information required from each site is a count of individuals per species. From the count data the communities can be characterized and compared according to community structure, density, biomass , diversity, or other analysis. Equally desirable is to characterize chemical and physical habitat such as dissolved oxygen concentration, substrate, water depth, type of sediment, grain size, total organic carbon, hardness, alkalinity, trace metals, dissolved and total nutrients, etc.

A. REFERENCES

Standard Methods for the Examination of Water and Wastewater (1992), Part 10500 Benthic Macroinvertebrates, at 10-55 to 10-65.

Techniques of Water-Resources Investigations of the USGS, Methods for Collection and Analysis of Aquatic Biological and Microbiological Samples, Book 5, Chapter A4, Benthic Invertebrates, at 151-189 (1987).